FEBS 29856 FEBS Letters 579 (2005) 4781–4786

The parkinsonism producing neurotoxin MPP^+ affects microtubule dynamics by acting as a destabilising factor

Graziella Cappelletti[a,*], Thomas Surrey[b], Rosalba Maci[a]

[a] *Department of Biology, University of Milano, Via Celoria, 26, 20133 Milano, Italy*
[b] *European Molecular Biology Laboratory (EMBL), Cell Biology and Biophysics Programme, Heidelberg, Meyerhofstrasse 1, 69117 – Heidelberg, Germany*

Received 20 May 2005; revised 8 July 2005; accepted 18 July 2005

Available online 8 August 2005

Edited by Jesus Avila

Abstract **Dysfunction of the microtubule system is emerging as a contributing factor in a number of neurodegenerative diseases. Looking for the potential role played by the microtubule cytoskeleton in neuron degeneration underlying Parkinson's disease (PD), we investigate the influence of the parkinsonism producing neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) on microtubule dynamics. We find that it acts as a strong catastrophe promoter causing a decrease of the average length of microtubules assembled from purified tubulin. We also find that it reduces the number of microtubules nucleated from purified centrosomes. Finally, binding assays demonstrate that the neurotoxin binds specifically to tubulin in the microtubule lattice in a close to stoichiometric manner. This paper provides the first evidence that dynamic instability of microtubules is specifically affected by MPP^+ and suggests that it could play a role in neuronal cell death underlying PD.**

Keywords: 1-Methyl-4-phenylpyridinium; Microtubule; Dynamics; Cytoskeleton; Neurodegeneration; Parkinson's disease

1. Introduction

The molecular pathways implicated in common neurodegenerative disorders are gradually being elucidated and several crucial events including accumulation of aberrant or misfolded proteins, mitochondrial injury, oxidative and nitrosative stress, and failure of axonal and dendritic transport are emerging [1]. Focusing on the dysfunction of the neuronal cytoskeleton, evidence is accumulating that a number of neurodegenerative diseases are characterised by typical abnormalities in the microtubule system [2,3]. However, little is known about cytoskeletal rearrangements involved in the onset of Parkinson's disease (PD). Tubulin is a major components of Lewy bodies, cytoplasmic inclusions typical of degenerated neurones in PD [4]. Recent papers on genetic parkinsonism support to the idea that the microtubular cytoskeleton could be a player in the neurodegenerative scenario. They suggest that a link between tau and PD exists [5] and that the stability of axonal microtubules could be directly affected by α-synuclein [6], which is the first mutated protein linked to PD [7]. They also show that parkin, which is linked to autosomal recessive juvenile PD [8], is a novel tubulin-binding protein, as well as a microtubule-associated protein [9,10].

We addressed the question of cytoskeleton rearrangements in PD neurodegeneration by using *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that kills dopamine neurons and induces PD-like symptoms [11]. It has therefore become an invaluable experimental tool for studies on sporadic PD [12]. We reported earlier that 1-methyl-4-phenylpyridinium (MPP^+), the toxic metabolite of MPTP, influences the state of tubulin polymerisation in PC12 cell line differentiated with nerve growth factor that is commonly used as a model for dopamine neurons [13,14]. The pool of unpolymerised tubulin was significantly increased and the synthesis rate was reduced in PC12 cells treated with sublethal concentrations of MPP^+. This corresponds exactly to the situation observed in HeLa cells following depletion of the microtubule-associated protein MAP4 [15] that is known to be an assembly promoting factor. Because microtubule-destabilising factors are defined as factors that cause a decrease in the total microtubule polymer mass when added to microtubules, the effects evoked by MPP^+ in PC12 cells could be the result of microtubule destabilisation. Looking for a direct effect of the neurotoxin on microtubules, we have undertaken an in vitro study by using tubulin purified from bovine brain and showed that MPP^+ inhibits tubulin polymerisation by lowering the initial rate and the final extent of assembly, and increasing the critical concentration for polymerisation [16]. In the present study, we have investigated the molecular mechanisms by which MPP^+ influences microtubule polymerisation dynamics. We analysed the effect of MPP^+ on the dynamic properties of microtubules assembled from purified tubulin by time-lapse video microscopy, and tested its ability to interact with dimeric tubulin or microtubules using a binding assay.

2. Materials and methods

2.1. Microtubule assembly assay

Calf brain tubulin and rhodamine-labelled tubulin were prepared according to Hyman et al. [17]. Pure tubulin (20 μM) was added to a reaction mixture containing 1.8 μM rhodamine-labelled tubulin, 1 μl

*Corresponding author. Fax: +39 02 50314802.
E-mail address: graziella.cappelletti@unimi.it (G. Cappelletti).

Abbreviations: DIC, differential interference contrast; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease

0014-5793/$30.00
doi:10.1016/j.febslet.2005.07.058

centrosomes (8×10^6/ml), 1 mM GTP in BRB80 (80 mM K-Pipes, pH 6.8, 1 mM EGTA, and 1 mM $MgCl_2$). Samples were incubated for 15 min at 37 °C or at room temperature in the presence of the indicated concentrations of MPTP or MPP^+ (RBI, Natick, MA, USA), and the assembled asters were fixed with 1 ml of 10% glycerol, 0.25% glutaraldehyde, 0.1% Triton X-100 in BRB80 and spun down onto coverslips. Images acquisition was performed using a Zeiss Axiovert 10 equipped with an 100x objective and a digital image recording system (Sony SSC M370CE CCD camera, Power Mac G3 and Scion Image 1.62 software). Microtubule lengths were determined using Scion Image.

2.2. Microtubule dynamics analysis

The behaviour of individual microtubules was monitored by video-enhanced differential interference contrast (VE-DIC) microscopy [18]. Centrosomes were diluted to 1×10^4/μl in BRB80, injected into a 4 μl perfusion chamber and left for 5 min on ice. The chamber was washed with 30 μl BRB80 and microtubule assembly was initiated by perfusion of 55 μM tubulin in BRB80 containing 1 mM GTP, 1 mM DTT, and increasing MPP^+ concentrations (always 6 μl total volume). Dynamics were observed for 40 min at 22 °C using an Olympus BX51 microscope equipped with DIC lens (Plan Apo 60x/1.4 numerical aperture) and video camera (Hamamatsu 2400 newvicon). Images were recorded every 2 s, enhanced with a Hamamatsu Argus 20 image processor and stored on a Macintosh using NIH Image software. Microtubules were measured in every frame to generate the graphs of microtubule lifetimes. Data analysis was done with a home-made Microsoft Excel macro to determine the growth rate and the shrinkage rate of microtubules [19]. Catastrophe frequency was calculated by dividing the total number of events by the duration of growth and pause phases.

2.3. Analysis of tubulin binding

We have measured the binding of MPP^+ to tubulin dimers by ultrafiltration and to microtubules by filtration. Tubulin dimers (25 μM) were incubated at 0° and 37 °C for 1 h in BRB80 containing 50 μM [^{3}H]-MPP^+ (0.2 Ci/mmol, Perkin–Elmer, Wellesley, MA, USA). Tubulin-MPP^+ complexes were separated from unbound MPP^+ by ultrafiltration using Microcon® YM-30 filters (Millipore Co., Bedford, MA, USA). The radioactivity was measured both in the retenate and in the ultrafiltrate. To confirm the presence of tubulin exclusively in the retenate, equivalent amounts of the retenate and ultrafiltate were analysed by 7.5% SDS–PAGE followed by Coomassie staining. Another set of experiments were performed on prepolymerised and stabilised microtubules. Here, microtubules (25 μM tubulin dimer), preincubated with 20 μM taxol for 15 min at 37 °C, were mixed with increasing MPP^+ concentrations (5–80 μM), [^{3}H]-MPP^+ (70 μCi/sample), 1 mM GTP, 20 μM taxol, in BRB80 and incubated for 15 min at 37 °C. Microtubules-MPP^+ complexes were separated from unbound MPP^+ and tubulin dimers by filtration using Ultrafree®-MC microporous filters (0.1 μm size, Millipore Co., Bedford, MA, USA). Aliquots of the retenate and ultrafiltrate were subjected to radioactivity measurement and analysed by 7.5% SDS–PAGE followed by Coomassie staining. In equilibrium binding experiments specific [^{3}H]-MPP^+ binding was calculated as the difference between total binding and non-specific binding occurring in the presence of an excess concentration of unlabelled MPP^+ (1000 times the concentration of labelled MPP^+). To calculate binding parameters, data were plotted according to the Scatchard equation.

2.4. Statistical analysis

The data were analysed using SigmaPlot 8.0 program (Systat Software Inc., Point Richmond, CA, USA). Difference between experiments with or without MPTP and MPP^+ were evaluated by a Student's *t*-test for unpaired data with a confidence level of 95%.

3. Results

In order to gain insight into the molecular mechanism by which MPP^+ influences microtubule polymerisation dynamics, we have undertaken in vitro experiments with pure tubulin and microtubules. First, we have evaluated the effect of the neuro-

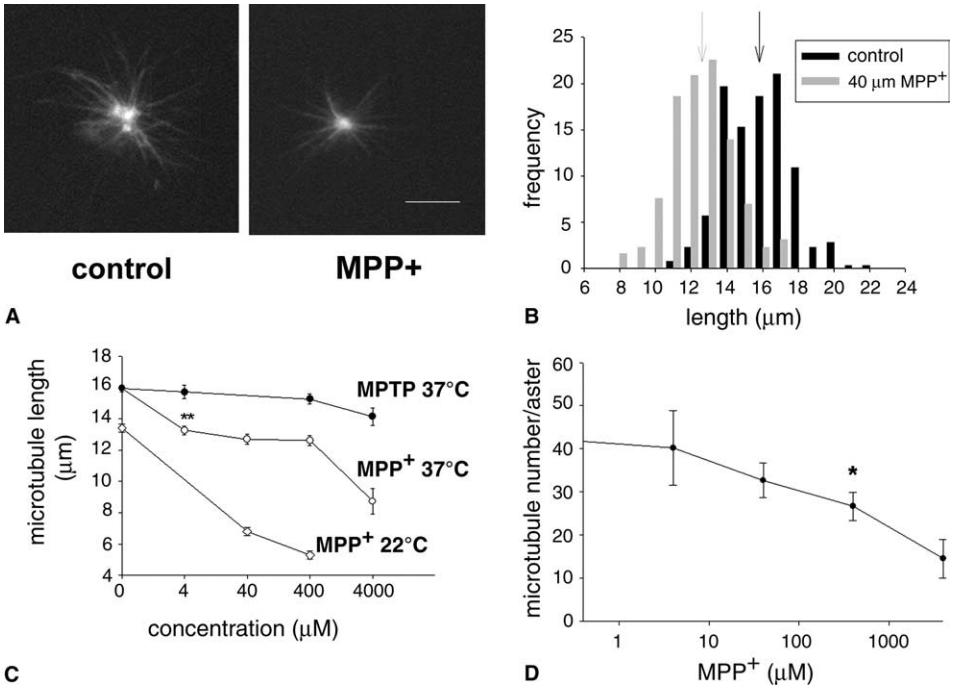

Fig. 1. MPP^+ affects the length and number of microtubules nucleated from centrosomes in vitro. (A) Microtubule asters nucleated from pure tubulin and centrosomes in the absence (control) or in the presence of 40 μM MPP^+. Bar, 10 μm. (B) Histogram of the frequencies of microtubule length in the absence (control) or in the presence of 40 μM MPP^+ obtained from four independent experiments. (C) Quantification of microtubule length in the presence of increasing concentrations of MPTP and MPP^+. Experiments were performed either at room temperature (22 °C) or at 37 °C as indicated in the figure. (D) Quantification of the number of microtubules polymerised per asters in the presence of increasing MPP^+ concentrations. Bars indicate S.E.M. $^*P < 0.05$; $^{**}P < 0.001$ according to Student's unpaired *t*-test.

toxin on aster formation and microtubule length. The presence of MPP^+ reduces the average length of microtubules nucleated from centrosomes after 20 min of incubation at 37 °C (Fig. 1A). We quantified the length of microtubules of the asters in the presence of increasing concentrations of MPP^+ and found a concentration-dependent decrease of the microtubule length (Fig. 1B and C). The MT destabilising effect of MPP^+ becomes significant at concentrations above 4 μM and it is stronger when tubulin polymerisation occurs at room temperature as compared to 37 °C. In agreement with our previous data showing that MPTP is ineffective on tubulin polymerisation in vitro [16], the length of microtubules appears to be unmodified by MPTP up to 4 mM. In addition to a decrease of the microtubule length, at higher concentrations of MPP^+ we also observed a significant decrease in the number of microtubule per aster (Fig. 1D). Therefore, we conclude that the presence of MPP^+ can influence both the average length of microtubules assembled from purified tubulin and the number of microtubules nucleated from purified centrosomes.

When individual microtubules are observed by light microscopy, both in vivo and in vitro, they display alternating phases of growth and shortening, collectively termed dynamic instability [20]. Due to this phenomenon, the steady-state length of an ensemble of microtubules results from the values of the rates of growth and shortening of the individual microtubules and from the average frequencies with which they convert between growth and shortening phases. To elucidate the molecular mechanism by which MPP^+ affects the steady-state length of microtubules, we monitored the real time behaviour of individual microtubules by VE-DIC microscopy [18]. Briefly, purified centrosomes were adsorbed to the surface of a perfusion chamber, and tubulin was perfused into the chamber in the absence or presence of increasing MPP^+ concentrations, and allowed to polymerise at room temperature. As expected, the extent of microtubule polymerisation was reduced with increasing MPP^+ concentrations (Fig. 2A). We analysed the life-history of individual microtubules polymerised in the absence or in the presence of MPP^+ (Fig. 2B). From such plots of the microtubule length versus time, we determined the values of all four parameters defining microtubule dynamic instability (Fig. 3). The most drastic effect of MPP^+ was observed on the frequency of transitions from growth to shrinkage, i.e., on the catastrophe frequency. It was significantly increased for microtubules polymerised in the presence of MPP^+ (Fig. 3A) in a concentration-dependent manner. We observed a 2-fold increase for microtubules polymerised in the presence of 4 μM MPP^+ up to a 20-fold increase at 400 μM MPP^+. On the contrary, the rescue frequency (Fig. 3B), the growth rate (Fig. 3C), and the shrinkage rate (Fig. 3D) were not strongly influenced by MPP^+. In detail, the rescue frequency was not significantly affected by low concentrations of MPP^+ and doubled at the highest concentration we tested (400 μM). The growth was reduced by up to a factor of 2 at 400 μM MPP^+ (0.6 ± 0.38 μm/min) with respect to the control (1.42 ± 0.42 μm/min), and the shrinkage rate was not affected at all. These results show that MPP^+ strongly influences microtubule dynamics, mostly by acting as a catastrophe promoter causing the observed decrease of the average length of microtubules.

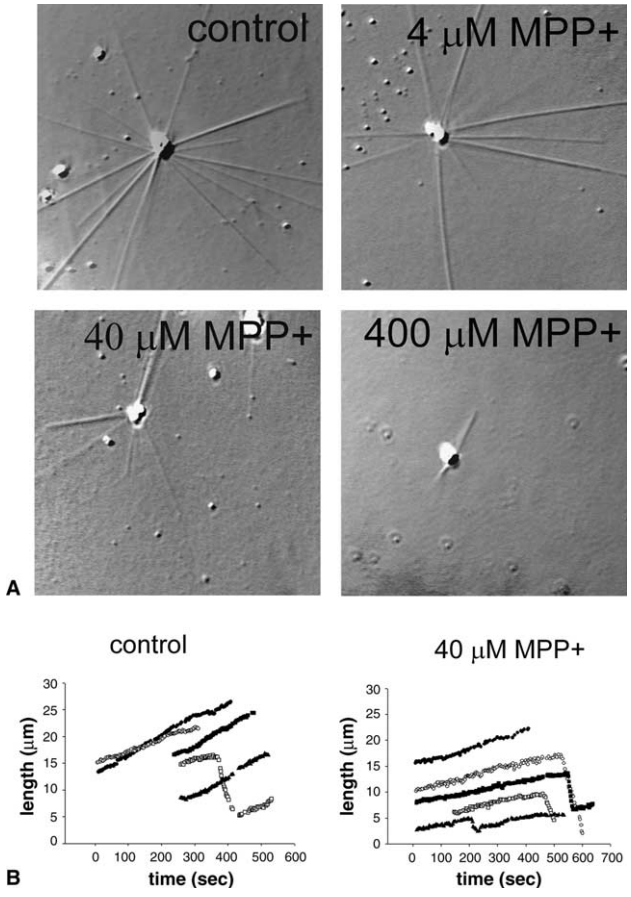

Fig. 2. MPP^+ affects microtubule dynamics. (A) Images of microtubules polymerised from centrosomes and tubulin in the absence (control) or in the presence of increasing MPP^+ concentrations and visualised after an incubation of 15 min at room temperature by DIC microscopy. Bar, 10 μM. (B) Life-history traces of microtubules polymerised in control buffer or in the presence of 40 μM MPP^+. Each plot displays the length of individual microtubules.

Microtubule dynamics can be modulated by the interaction of regulatory molecules with soluble tubulin or by binding of regulatory molecules to distinct binding sites at the microtubule surface or end. To learn more about the mechanism of the MPP^+-induced effect on microtubule dynamics, we tested its ability to bind dimeric tubulin or microtubules. We incubated free tubulin or taxol-stabilised microtubules with [^{3}H]-MPP^+ and separated the bound fraction of MPP^+ from the unbound fraction by ultrafiltration. We found no radioactivity associated with free tubulin showing that MPP^+ does not strongly bind to dimeric tubulin (Fig. 4A). Using this assay, we cannot rule out, however, that MPP^+ might bind weakly to dimeric tubulin. In contrast, a high amount of [^{3}H]-MPP^+ was found to be associated to polymerised tubulin showing that MPP^+ strongly binds to microtubules (Fig. 4B). Next, we investigated whether the binding to microtubules was concentration-dependent. Fig. 4C shows the results of equilibrium binding studies in which the binding shows saturation behaviour with increasing concentrations of [^{3}H]-MPP^+. Specific [^{3}H]-MPP^+ binding was calculated as the difference between total binding and non-specific binding occurring in the presence of an excess concentration of unlabelled MPP^+ (1000 times the concentration of labelled MPP^+). To calculate binding parameters, we analysed the data according to Scatchard (see Section 2). The Scatchard plot for the specific [^{3}H]-MPP^+ binding was linear (Fig. 4D), indicating the presence

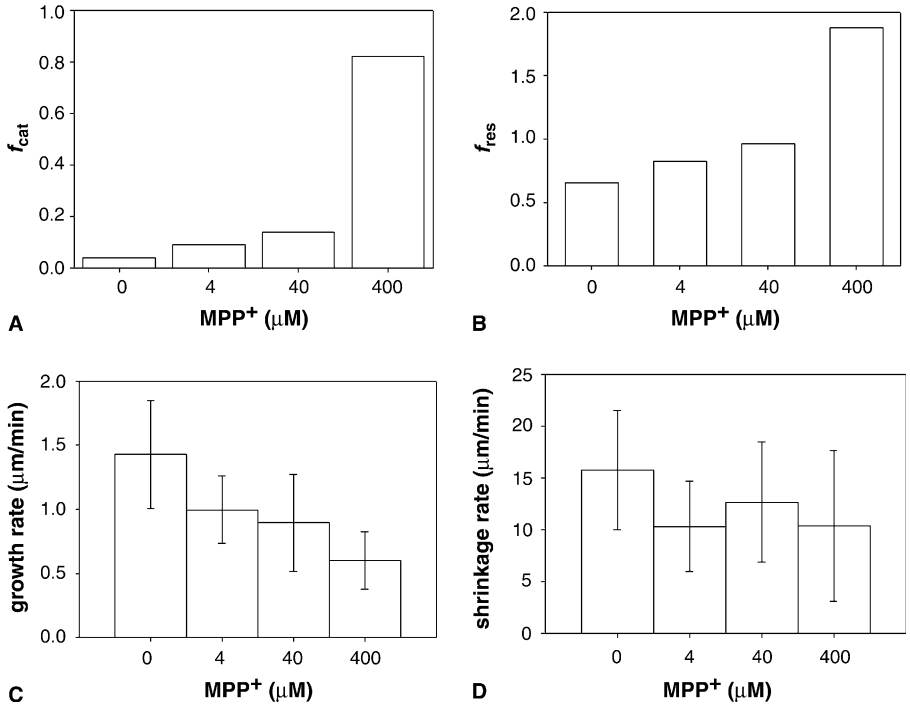

Fig. 3. MPP^+ induces microtubule catastrophe. We measured the parameters of microtubule dynamic instability from the movies recordered over time of microtubule asters nucleated in the presence of increasing concentrations of MPP^+. (A) The catastrophe frequency (f_{cat}), (B) the rescue frequency (f_{res}), (C) the growth rate, and (D) the shrinkage rate were determined by analysing 51 microtubules for control, 31 microtubules for 4 μM MPP^+, 32 microtubules for 40 μM MPP^+ and 7 microtubules for 400 μM MPP^+. Bars indicate S.E.M.

of a single class of binding sites, and its regression analysis ($r = 0.96$) yields an equilibrium dissociation constant $K_D = 46$ μM and the number of binding site per molecule of tubulin $n = 0.49$. These results indicate that MPP^+ specifically binds to tubulin in the microtubule lattice close to stoichiometric manner.

4. Discussion

The present study provides the first evidence that the parkinsonism-producing neurotoxin MPP^+ specifically affects microtubule dynamics in vitro. Microtubules are dynamic polymers that can transit stochastically between growing and shrinking states, a behaviour known as dynamic instability [20]. We show that MPP^+ reduces the average length and the number of microtubules nucleated from centrosomes. It does so by acting as a catastrophe promoting factor, i.e., by increasing the frequency of transitions from microtubule growth to shrinkage. We finally show that MPP^+ binds specifically to tubulin in the microtubule lattice in a close to stoichiometric manner. It is interesting to note that the concentrations of MPP^+ inducing such a strong effect on microtubule dynamics in vitro are similar to those required to induce neurotoxicity in neuronal cells expressing dopamine transporter [13,36]. We suggest that microtubules might be a novel target in MPP^+-induced neurodegeneration.

The effect of MPP^+ on the catastrophe frequency might partly be a result of the decreased growth rate as previously described [21]. However, at MPP^+ concentrations well above its K_D we find that the increase of the catastrophe frequency is much more pronounced than the decrease of the growth rate that is still in a range usually not strongly affecting the catastrophe frequency. Therefore, MPP^+ most likely has a specific effect on the catastrophe frequency. Comparing MPP^+ to microtubule-destabilising proteins (Stathmin/Op 18, KinI/XKCM1) that regulate microtubule dynamics by increasing the catastrophe frequency [22,23], we note a difference in the apparent mechanism by which MPP^+ and these catastrophe promoting proteins act. Our binding experiments show that the neurotoxin specifically binds to tubulin along the microtubule lattice in a close to stoichiometric manner and suggest that there is one MPP^+ binding site for every two tubulin heterodimers. This means that MPP^+ regulates the dynamics through the interaction with a large number of distinct tubulin sites on microtubules. The usual mechanism of action, however, proposed for microtubule-destabilising proteins or drugs involves either the sequestration of tubulin dimers to prevent their assembly or the specific interaction with microtubule tips [24,25]. The stoichiometric binding of MPP^+ along the microtubule surface appears therefore to represent a mechanism leading to microtubule destabilisation. This mode of action is reminiscent of the action of a kinesin-like protein (XKLP1) that binds only weakly all along the microtubules, but nevertheless alters their dynamic properties, most likely through a structural change in the microtubule lattice [27] or even of vinblastine, a known tubulin-targeting alkaloid that binds to polymerised tubulin [26]. The structural basis for the regulation of tubulin by vinbastine has only recently been discovered by X-ray analysis of vinblastine bound to tubulin in a complex with the RB3 showing that it binds at the

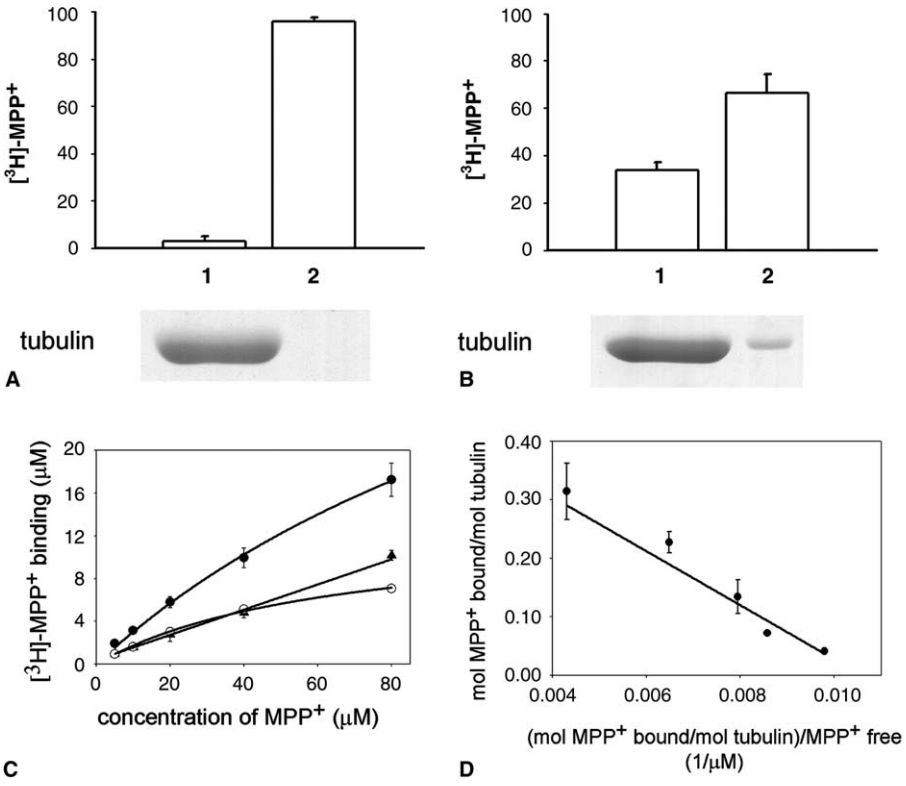

Fig. 4. MPP^+ binds to microtubules. (A) Tubulin dimers (25 μM) were incubated in the presence of [^{3}H]-MPP^+ (50 μM) for 1 h on ice and then ultrafiltrated (Section 2). A Coomassie blue-stained gel shows that tubulin was entirely recovered in the retenate (1). The radioactivity was measured both in the retenate (1) and in the ultrafiltrate (2), and expressed as percentage of total radioactivity in the assay. (B) Microtubules polymerised from pure tubulin (25 μM) in the presence 20 μM taxol were incubated for 1 h at 37 °C in the presence of [^{3}H]-MPP^+ (50 μM) and then filtrated (Section 2). The Coomassie Blue-stained gel shows that 90% of tubulin was recovered in the retenate (microtubules) and 10% in the ultrafiltrate (tubulin dimers). The radioactivity was measured both in the retenate (1) and in the filtrate (2), and expressed as percentage of total radioactivity in the assay. (C) Binding of [^{3}H]-MPP^+ to microtubules. ●, total binding; ▲, non-specific binding; ○, saturable binding, the difference between the total and non-specific values. Bars indicate S.E.M. (D) Scatchard plot of the saturable binding.

interface of two heterodimers [37] and suggesting that intermolecular contact in microtubules could be an attractive target for microtubule perturbing drugs.

A growing body of evidence indicates that the interaction with and the subsequent dysfunction of the microtubule cytoskeleton could be common to other toxins known to cause PD and to proteins mutated in PD. Gearhart et al. [28] identified α-tubulin as one of the brain proteins interacting with an environmental toxin analog of MPP^+ by phage display. In addition, rotenone, whose administration leads to selective degeneration of nigral neurons and parkinsonism [29], binds tubulin and potently depolymerises microtubules in vivo and in vitro [30]. Further evidence arises from studies on mutated genes in parkinsonism. It has been shown that α-synuclein is associated with microtubules by copurification from rat brain and that tubulin initiates and promotes α-synuclein fibril formation under physiological conditions in vitro suggesting that α-synuclein could function as a microtubule-associated protein and, consequently, be implicated in the regulation of microtubule-dependent neuronal functions [6]. More recent studies have shown that parkin is a novel tubulin binding protein that enhances the ubiquitination and degradation of misfolded tubulin [9,10], as well as a γ-tubulin interacting protein [31]. Here, our results show that MPP^+, the toxic metabolite of MPTP, binds microtubules and affects microtubule stability. In conclusions, the notion that tubulin might be an interaction partner for numerous players in neurodegeneration seems to become a well supported hypothesis.

It has been recently proposed that there is a limited range of acceptable microtubule dynamic behaviours in neurons, outside of which microtubules cannot function normally and the cells cannot survive [32]. Among the microtubule-dependent functions axonal transport could be a good candidate to explain how a cell could die following microtubule dysfunction. Although the underlying mechanism connecting microtubule dynamics to axonal transport is unclear, treatment of cells with taxol at levels causing a modest overstabilisation of microtubules has been shown to compromise axonal transport [33] as well as the elevation of tau, known for its role in the stabilisation of microtubules, causes transport defects [34]. Another possibility is that the interference with the dynamics of axonal microtubules could drastically compromise synaptic functionality. This would agree with previous data reporting that the dynamic instability of microtubules at the distal axon may also contribute to the release of vescicles from the microtubules and to the insertion of new membranes into the nerve ends [35]. All these data support the hypothesis that the change of microtubule dynamics as evoked by MPP^+ might be a pathogenic mechanism leading to neuronal cell death.

Acknowledgements: We thank Eric Karsenti (EMBL, Heidelberg, Germany) for supporting this project and for helpful discussions. We also thank Isabelle Arnal (Universite de Rennes 1, Rennes Cedex, France) for an introduction into the analysis of microtubule dynamics. This work was supported by a fellowship to GC from EurALMF (European Advanced Light Microscopy Facility of EMBL, Heidelberg, Germany), and funding from MIUR (FIRB Grant No. RBAUO1FSR9 to RM) and the University of Milano (FIRST 2003–2004).

References

[1] Bossy-Wetzel, E., Schwarzenbacher, R. and Lipton, S.L. (2004) Molecular pathways to neurodegeneration. Nat. Med. 10, S2–S9.
[2] Crosby, A.H. (2003) Disruption of cellular transport: a common cause of neurodegeneration? Lancet Neurol. 2, 311–316.
[3] Avila, J., Lucas, J.J., Pèrez, M. and Hernandez, F. (2004) Role of tau in both physiological and pathological conditions. Physiol. Rev. 84, 361–384.
[4] Galloway, P.P., Mulvihill, P. and Perry, G. (1992) Filaments of Lewy bodies contain insoluble cytoskeletal elements. Am. J. Pathol. 140, 809–822.
[5] Spillantini, M.G. and Goedert, M. (2001) Tau and Parkinson's disease. JAMA 286, 2324–2326.
[6] Alim, M.A., Hossain, M.S., Arima, K., Takeda, K., Izumiyama, Y., Nakamura, M., Kaji, H., Shinoda, T., Hisanaga, S. and Ueda, K. (2002) Tubulin seeds α-synuclein fibril formation. J. Biol. Chem. 277, 2112–2117.
[7] Polymeropoulos, M.H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J. and Boyer, R., et al. (1997) Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science 276, 2045–2047.
[8] Kitada, T., Asakawa, S., Matsumine, H., Yamamura, Y., Minoshima, S., Yokoshi, M., Mizuno, Y. and Shimizu, N. (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392, 605–608.
[9] Ren, Y., Zhao, J. and Feng, J. (2003) Parkin binds to α/β tubulin and increases their ubiquitination and degradation. J. Neurosci. 23 (8), 3316–3324.
[10] Yang, F., Jiang, Q., Zhao, J., Ren, Y., Sutton, M.D. and Feng, J. (2005) Parkin stabilizes microtubules through strong binding mediated by three independent domains. J. Biol. Chem. 280, 17154–17162.
[11] Langston, J.W., Ballard, P., Tetrud, J.W. and Irwin, I. (1983) Chronic Parkinsonism due to a product of meperidine-analog synthesis. Science 219, 979–980.
[12] Beal, M.F. (2001) Experimental models of Parkinson's disease. Nat. Rev. Neurosci. 2, 325–332.
[13] Cappelletti, G., Maggioni, M.G. and Maci, R. (1999) Influence of MPP^+ on the state of tubulin polymerisation in NGF-differentiated PC12 cells. J. Neurosci. Res. 56, 28–35.
[14] Cappelletti, G., Maggioni, M.G. and Maci, R. (2000) Role of microtubules in the genesis of MPTP neurotoxicity in: Neurotoxic Factors in Parkinson's Disease and Related Disorder (Storch, A. and Collins, M.A., Eds.), pp. 45–48, Kluwer Academic/Plenum Publishers, New York.
[15] Nguyen, H.L., Gruber, D. and Bulinski, J.C. (1999) Microtubule-associated protein 4 (MAP4) regulates assembly, protomer-polymer partitioning and synthesis of tubulin in cultured cells. J. Cell Sci. 112 (Pt 12), 1813–1824.
[16] Cappelletti, G., Pedrotti, B., Maggioni, M.G. and Maci, R. (2001) Tubulin polymerisation is directly affected by MPP^+ in vitro. Cell Biol. Int. 25, 981–984.
[17] Hyman, A., Drechsel, D., Kellogg, D., Salser, S., Sawin, K., Steffen, P., Wordeman, L. and Mitchison, T. (1991) Preparation of modified tubulins. Methods Enzymol. 196, 478–485.
[18] Kinoshita, K., Arnal, I., Desai, A., Drechsel, D.N. and Hyman, A.A. (2001) Reconstitution of physiological microtubule dynamics using purified components. Science 294, 1340–1343.
[19] Tournebize, R., Andersen, S.S.L., Verde, F., Dorèe, M., Karsenti, E. and Hyman, A.A. (1997) Distinct roles of PP1 and PP2A-like phosphatases in control of microtubule dynamics during mitosis. EMBO J. 16, 5537–5549.
[20] Mitchison, T. and Kirschner, M. (1984) Dynamic instability of microtubules growth. Nature 312, 237–242.
[21] Drechsel, D.N., Hyman, A.A., Cobb, M.H. and Kirschner, M.W. (1992) Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. Mol. Biol. Cell 3, 1141–1154.
[22] Belmont, L. and Mitchison, T.J. (1996) Identification of a protein that interacts with tubulin dimers and increases the catastrophe rate of microtubules. Cell 84, 623–631.
[23] Walczak, C.E., Mitchison, T.J. and Desai, A. (1996) XKCM1: a Xenopus kinesin-related protein that regulates microtubule dynamics during mitotic spindle assembly. Cell 84, 37–47.
[24] Curmi, P.A., Andersen, S.S.L., Lachkar, S., Gavet, O., Karsenti, E., Knossow, M. and Sobel, A. (1997) The stathmin/tubulin interaction in vitro. J. Biol. Chem. 272, 25029–25036.
[25] Jordan, M.A. and Wilson, L. (2004) Microtubules as a target for anticancer drugs. Nat. Rev. Cancer 4, 253–265.
[26] Singer, W.D., Jordan, M.A., Wilson, L. and Himes, R.H. (1989) Binding of vinblastine to stabilized microtubules. Mol. Pharmacol. 36, 366–370.
[27] Bringmann, H., Skinitis, G., Spilker, A., Kandels-Lewis, I., Vernos, I. and Surrey, T. (2004) A kinesin-like motor inhibits microtubule dynamic instability. Science 303, 1519–1522.
[28] Gearhart, D.A., Toole, P.F. and Beach, J.W. (2002) Identification of brain proteins that interact with 2-methylnorharman, an analog of the parkinsonian-inducing toxin, MPP^+. Neurosci. Res. 44, 255–265.
[29] Betarbet, R., Sherer, T.B., MacKenzie, G., Garcia-Osuna, M., Panov, A.V. and Greenamyre, J.T. (2000) Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat. Neurosci. 3 (12), 1301–1306.
[30] Brinkley, B.R., Barham, S.S., Barranco, S.C. and Fuller, G.M. (1974) Rotenone inhibition of spindle microtubule assembly in mammalian cells. Exp. Cell Res. 85, 41–46.
[31] Zhao, J., Ren, Y., Jiang, Q. and Feng, J. (2003) Parkin is recruited to the centrosome in response to inhibition of proteasome. J. Cell Sci. 116, 4011–4019.
[32] Feinstein, S.C. and Wilson, L. (2005) Inability of tau to properly regulate neuronal microtubule dynamics: loss-of-function mechanism by which tau might mediate neuronal cell death. Biochim. Biophys. Acta 1739 (2–3), 268–279.
[33] Nakata, T. and Hirokawa, N. (2003) Microtubules provide directional clues for polarized axonal transport through interaction with kinesin motor head. J. Cell Biol. 162, 1045–1055.
[34] Stamer, K., Vogel, R., Thies, E., Mandelkow, E. and Mandelkow, E.-M. (2002) Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. J. Cell Biol. 156, 1051–1063.
[35] Zakharenko, S. and Popov, S. (1998) Dynamics of axonal microtubules regulate the topology of new membrane insertion into the growing neurites. J. Cell Biol. 143, 1077–1086.
[36] Heider, I., Lehmensiek, V., Lenk, T., Mullerm, T. and Storch, A. (2004) Dopaminergic neurotoxicity of homocysteine and its derivatives in primary mesencephalic cultures. J. Neural Transm. Suppl. 68, 1–13.
[37] Gigant, B., Wang, C., Ravelli, R.B.G., Roussi, F., Steinmetz, M.O., Curmi, P.A, Sobel, A. and Knossow, M. (2005) Structural basis for the regulation of tubulin by vinblastine. Nature 435, 519–522.